Claims

1. A substantially pure heparinase comprising:

a modified heparinase II having a modified product profile, wherein the modified product profile of the modified heparinase II is at least 10% different than a native product profile of a native heparinase II.

2. The heparinase of claim 1, wherein the modified heparinase II has a modified product profile that is at least 50% different than a native product profile of a native heparinase II.

3. The heparinase of claim 1, wherein the modified heparinase II has a modified product profile that is at least 20% different than a native product profile of a native heparinase II.

4. The heparinase of claim 1, wherein the modified product profile is modified with respect to heparin.

5. The heparinase of claim 1, wherein the modified product profile is modified with respect to heparan sulfate.

6. A pharmaceutical preparation comprising a sterile formulation of the substantially pure heparinase of claim 1 and a pharmaceutically acceptable carrier.

7. An immobilized substantially pure modified heparinase II comprising:

a modified heparinase II as in claim 1, and

a solid support membrane, wherein the modified heparinase II is immobilized on the solid support membrane.

8. A substantially pure heparinase comprising:

a modified heparinase II that can cleave a glycosaminoglycan substrate having a modified heparinase II k_{cat} value, wherein the modified heparinase II k_{cat} value is at least 10% different than a native heparinase II k_{cat} value.

9. The heparinase of claim 8, wherein the modified heparinase II k_{cat} value is at least 20% different than a native heparinase II k_{cat} value.

10. The heparinase of claim 8, wherein the modified heparinase II k_{cat} value is at least 50% different than a native heparinase II k_{cat} value.

11. The heparinase of claim 8, wherein the modified heparinase II has a reduced enzymatic activity with respect to heparin.

12. The heparinase of claim 8, wherein the modified heparinase II has a reduced enzymatic activity with respect to heparan sulfate.

13. The heparinase of claim 8, wherein the modified heparinase II has the amino acid sequence of the mature peptide of SEQ ID NO: 2 wherein at least one amino acid residue has been substituted and wherein the substitution is selected from the group consisting of (a) a substitution of a cysteine residue corresponding to position 348 of SEQ ID NO: 2 with a

residue selected from the group consisting of alanine, serine, tyrosine, histidine, threonine, and lysine; (b) a substitution of a histidine residue corresponding to at least one of positions 238, 440, 451, and 579 of SEQ ID NO: 2 with a residue selected from the group consisting of alanine, serine, tyrosine, threonine, and lysine; and (c) a conservative substitution of a heparin-binding sequence corresponding to positions 446-451 of SEQ ID NO: 2.

14. The heparinase of claim 13, wherein the modified heparinase II has the amino acid sequence of the mature peptide of SEQ ID NO: 2 wherein the cysteine residue corresponding to position 348 of SEQ ID NO: 2 has been substituted with a residue selected from the group consisting of alanine, serine, tyrosine, histidine, threonine, and lysine.

15. The heparinase of claim 14, wherein the cysteine residue has been substituted with an alanine.

16. The heparinase of claim 13, wherein the modified heparinase II has the amino acid sequence of the mature peptide of SEQ ID NO: 2 wherein the histidine residue corresponding to position 440 of SEQ ID NO: 2 has been substituted with a residue selected from the group consisting of alanine, serine, tyrosine, threonine, and lysine.

17. The heparinase of claim 16, wherein the histidine residue has been substituted with an alanine.

18. A pharmaceutical preparation comprising a sterile formulation of the substantially pure heparinase of claim 8 and a pharmaceutically acceptable carrier.

19. An immobilized substantially pure modified heparinase II comprising:

a modified heparinase II as in claim 8, and

a solid support membrane, wherein the modified heparinase II is immobilized on the solid support membrane.

20. The heparinase of claim 11, wherein the modified heparinase II has substantially the same enzymatic activity as native heparinase with respect to heparan sulfate.

21. The heparinase of claim 12, wherein the modified heparinase II has substantially the same enzymatic activity as native heparinase with respect to heparin.

22. A substantially pure heparinase comprising:

a modified heparinase I wherein the modified heparinase I has enzymatic activity that is not dependent on the presence of calcium.

23. The heparinase of claim 22, wherein the modified heparinase I has a modified heparinase I k_{cat} value that is at least 10% different than a native heparinase I k_{cat} value.

24. The heparinase of claim 22, wherein the modified heparinase I has a modified heparinase I k_{cat} value that is at least 20% different than a native heparinase I k_{cat} value.

25. The heparinase of claim 22, wherein the modified heparinase I has a modified heparinase I k_{cat} value that is at least 50% different than a native heparinase I k_{cat} value.

26. The heparinase of claim 22, wherein the modified heparinase I has the amino acid sequence of the mature peptide of SEQ ID NO: 4 wherein at least one amino acid residue has been substituted and wherein the substitution is a substitution of a serine residue corresponding to position 377 of SEQ ID NO: 4 with a residue selected from the group consisting of alanine, serine, tyrosine, histidine, threonine, and lysine.

27. The heparinase of claim 26, wherein the serine residue has been substituted with an alanine.

28. A pharmaceutical preparation comprising a sterile formulation of the substantially pure heparinase of claim 22 and a pharmaceutically acceptable carrier.

29. An immobilized substantially pure modified heparinase I comprising:

a modified heparinase I as in claim 22, and

a solid support membrane, wherein the modified heparinase I is immobilized on the solid support membrane.

30. A method of specifically cleaving a heparin-like glycosaminoglycan, comprising:

contacting a heparin-like glycosaminoglycan with the heparinase of any one of claims 1, 8, or 22.

31. The method of claim 30, wherein the heparin-like glycosaminoglycan is contacted with a modified heparinase II, wherein the modified heparinase II has the amino acid sequence

of the mature peptide of SEQ ID NO: 2 wherein the histidine residue corresponding to position 440 of SEQ. ID NO: 2 is substituted with a residue selected from the group consisting of alanine, serine, tyrosine, threonine, and lysine to specifically cleave a heparin-like glycosaminoglycan.

32. The method of claim 30, wherein the heparin-like glycosaminoglycan is contacted with a modified heparinase I, wherein the modified heparinase I has the amino acid sequence of the mature peptide of SEQ ID NO: 4 wherein at least one amino acid residue has been substituted and wherein the substitution is a substitution of a serine residue corresponding to position 377 of SEQ ID NO: 4 with a residue selected from the group consisting of alanine, serine, tyrosine, histidine, threonine, and lysine.

33. The method of claims 30, wherein the method is a method of removing active heparin from a heparin containing fluid.

34. The method of claim 33, wherein the heparinase is immobilized on a solid support.

35. The method of claim 30, wherein the method is a method for inhibiting angiogenesis and wherein an effective amount for inhibiting angiogenesis of the heparinase is administered to a subject in need of treatment thereof.

36. The method of claim 35 wherein the heparinase is administered to a tumor.

37. The method of claim 35, wherein the heparinase is administered in a biodegradable, biocompatible polymeric delivery device.

38. The method of claim 35, wherein the heparinase is administered in a pharmaceutically acceptable vehicle for injection.

39. The method of any one of claims 37 or 38, wherein the heparinase is administered in an effective amount for diminishing the number of blood vessels growing into a tumor.

40. The method of claim 35, wherein the heparinase is administered in a pharmaceutically acceptable vehicle for topical application to the eye.

41. The method of claim 40, wherein the heparinase is administered in an effective amount for diminishing the symptoms of an eye disease characterized by abnormal neovascularization.

42. The method of claim 35, wherein the heparinase is administered in a pharmaceutical vehicle suitable for topical application.

43. The method of claim 42, wherein the heparinase is administered in an effective amount for diminishing the symptoms of psoriasis.

44. The method of claim 35, wherein an effective amount for inhibiting angiogenesis is between approximately one and four μg heparinase or a concentration of between 10 and 100 nM heparinase.

45. The method of claim 30, wherein the method is a method for sequencing heparin.

46. A method of specifically cleaving a heparan sulfate-like glycosaminoglycan, comprising:

contacting a heparan sulfate containing fluid with the heparinase of any one of claims 1 or 8.

47. The method of claim 46, wherein the method is a method of removing active heparan sulfate from a heparan sulfate containing fluid.

48. The method of claim 47 wherein the heparinase is immobilized on a solid support.

49. The method of claim 46, wherein the heparan sulfate-like glycosaminoglycan is contacted with a substantially pure modified heparinase II, wherein the modified heparinase II has the amino acid sequence of the mature peptide of SEQ ID NO: 2 wherein the cysteine residue corresponding to position 348 of SEQ ID NO: 2 has been substituted with a residue selected from the group consisting of alanine, serine, tyrosine, histidine, threonine, and lysine to specifically cleave a heparin sulfate-like glycosaminoglycan.

50. The method of claim 49, wherein the method is a method for inhibiting cellular proliferation.

51. The method of claim 46, wherein the method is a method for sequencing heparan sulfate.

52. A substantially pure heparinase, comprising:

a polypeptide having the amino acid sequence of the mature peptide of SEQ ID NO: 2 wherein at least one amino acid residue has been substituted and wherein the substitution is selected from the group consisting of (a) a substitution of a cysteine residue corresponding to position 348 of SEQ ID NO: 2 with a residue selected from the group consisting of alanine, serine, tyrosine, histidine, threonine, and lysine; (b) a substitution of a histidine residue corresponding to position 440 of SEQ ID NO: 2 with a residue selected from the group consisting of alanine, serine, tyrosine, threonine, and lysine; and (c) a conservative substitution of a heparin-binding sequence corresponding to positions 446-451 of SEQ ID NO: 2.

53. A pharmaceutical preparation comprising a sterile formulation of the heparinase of claim 52 and a pharmaceutically acceptable carrier.

54. An immobilized substantially pure modified heparinase II comprising:

a heparinase as in claim 52, and

a solid support membrane, wherein the heparinase is immobilized on the solid support membrane.

55. An isolated nucleic acid comprising

(a) an isolated nucleic acid encoding the substantially pure heparinase of claim 52;

(b) nucleic acids which hybridize under stringent hybridization conditions to the nucleic acid of SEQ ID NO 1 or to the complement of the nucleic acid of SEQ ID NO 1 and which are modified to encode a modified heparinase as described in claim 52; and

(c) nucleic acids that differ from the nucleic acids of (b) in codon sequence due to the degeneracy of the genetic code.

56. A recombinant host cell including an isolated nucleic acid as in claim 55.

57. An expression vector including an isolated nucleic acid as in claim 55.